EXHIBIT DD

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BIOSYNTHESIS AND REGULATION OF IMMUNOGLOBULINS

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INTRODUCTION

Immunoglobulin gene expression exhibits a number of intriguing regulatory features. Immunoglobulin genes undergo dynamic DNA rearrangements and somatic mutations. The expression of immunoglobulin genes exhibits allelic exclusion and isotypic exclusion. The developmental regulation of immunoglobulin gene expression during B cell differentiation is characterized by (*a*) the differential onset of heavy and light chain production, (*b*) great changes in the level of expression, and (*c*) transition from the insertion of immunoglobulin as antigen receptors in the lymphocyte membrane to its active secretion.

Darnell (40, 41) and Brown (27) have recently reviewed the variety of transcriptional, post-transcriptional (e.g. RNA processing), and translational mechanisms employed in the regulation of eukaryotic gene expression. This review summarizes recent progress towards understanding the molecular mechanisms in immunoglobulin gene expression and regulation in the context of the developments in other eukaryotic genes. The complex array of lymphoid cell and lymphokine factor interactions that affect immunoglobulin gene expression are not considered here.

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IMMUNOGLOBULIN STRUCTURE

Most immunoglobulin molecules consist of two identical light (L) chains and two identical heavy (H) chains (74). Immunoglobulin L and H chains are encoded by three unlinked gene families—one each for κ and λ L chains, and one for H chains—which are present on mouse chromosomes 6, 16, and 12, respectively (reviewed in 43). Each chain is composed of a series of homologous domains, the NH_2-terminal domain is the variable (V) region and the remainder form the constant (C) region. The combination of H and L-chain V regions are responsible for antigen binding and may include thousands of different sequences. In contrast to V regions, the C regions have only a few alternative sequences. The various classes (IgM, IgD, IgG, IgE, IgA) and subclasses (e.g. IgG1, IgG2, IgG3, IgG4) of immunoglobulins with different biological functions are distinguished by different heavy chains (μ, δ, γ, ϵ, α) defined by their C regions (C_μ, C_δ, C_γ, C_ϵ, C_α). All classes of immunoglobulin contain light chains of either κ or λ isotype (containing C_κ or C_λ regions).

The immunoglobulin (Ig) molecule can exist in two very different environments: in the lymphocyte cell membrane as a surface antigen receptor, and in the circulation as a secreted antibody. For most immunoglobulin classes, the Ig molecules are secreted as four-chain disulfide-linked monomers (H_2L_2). However for IgM and IgA, the heavy chains carry a short COOH-terminal sequence, which permits polymerization of the monomers. These polymeric immunoglobulin molecules are covalently linked by disulfide bonds from their H-chain COOH-terminal segments to a single J (or joining) chain molecule per IgM or IgA polymer (85, 100). Thus, IgM is secreted as a pentamer $(\mu_2L_2)_5J$ and IgA can be secreted as a monomer (α_2L_2), dimer $(\alpha_2L_2)_2J$, or trimer $(\alpha_2L_2)_3J$.

Immunologists have long known that the proliferation and maturation of B lymphocytes in response to antigen (and perhaps other external signals such as anti-idiotypic antibody) is triggered by binding of antigen to membrane Ig displayed on the surfaces of B cells. It is likely that all classes and subclasses of Ig can exist in a membrane form as well as a secreted form. In contrast to secreted Ig molecules, all membrane Ig molecules, including IgM and IgA, are present as monomeric structures (H_2L_2) (110). Studies from a number of laboratories have demonstrated that membrane μ (μ_m) and secreted μ (μ_s) chains have different molecular properties. As would be expected for integral membrane proteins, μ_m chains exhibit hydrophobic behavior (123, 176). Protein and nucleic acid structural studies demonstrate identical sequences up to their COOH-terminal segments, but unique COOH-terminal sequences for both μ_s and μ_m H chains (2, 48, 77, 136, 196). More recent studies have shown different membrane and secreted

species for murine γ_3, γ_1, γ_{2a}, γ_{2b}, δ, and α H chains (58, 82, 88, 97, 102, 112, 116, 121, 159, 188). The COOH-terminal sequences of the membrane forms of γ_3, γ_1, γ_{2a}, γ_{2b}, δ, and α H chains have been inferred from nucleic acid sequences of cDNA or genomic DNA clones (33, 125, 140, 171, 189, 193). The COOH-terminal sequence of the membrane forms of H chains includes (*a*) an acidic segment of 12–25 amino acids, which has a net charge of –6; (*b*) a highly conserved sequence of 26 amino acids, which is designated the hydrophobic segment and is thought to anchor the H chain into the plasma membrane; and (*c*) an intracellular segment of variable length, which begins with the residues Lys-Val-Lys. The transmembrane peptide sequences of all H chain M exons show an unexpectedly high level of sequence conservation. This sequence conservation is the basis of a two-chain model for transmembrane insertion of surface immunoglobulins (136, 140). The short, positively charged intracellular sequence constitutes the complete intracellular segment of μ and δ H chains, whereas γ_1, γ_{2b}, and γ_{2a} (and presumably γ_3) H chains have conserved intracellular segments of 28 residues, which include the Lys-Val-Lys sequence. It is not known if the different intracellular segments exert different activation functions in cells expressing membrane IgG or IgA as compared to cells expressing membrane IgM or IgD.

DIFFERENTIAL IMMUNOGLOBULIN EXPRESSION DURING B-LYMPHOCYTE DEVELOPMENT

The developmental stages in B-lymphocyte development into Ig-secreting plasma cells are distinguished by differences in the organization of H and L-chain genes, the kinds of Ig chains expressed, and the levels of Ig gene expression (see Figure 1). It appears that a pluripotential hematopoietic stem cell generates lymphoid stem cells that subsequently generate pre-B cells, i.e. the first stage of B cell development in which cells express immunoglobulin chains (29, 95, 154). As a lymphoid stem cell differentiates to a pre-B cell, there is μ H chain gene rearrangement, transcription, translation, and expression of cytoplasmic μ H chains, but no expression of L chains. Early pre-B cells have not rearranged their L-chain genes, but are proliferating cells presumed eventually to rearrange and express different L-chain genes together with the H chain to which the pre-B cell is already committed (29, 95, 134b). A possible late pre-B cell developmental stage is suggested by several recent examples of pre-B-cell lines that have functionally rearranged κ L chain genes, but do not transcribe L chain mRNA until the cells are stimulated by bacterial lipopolysaccharide (101a, 128, 142a). This presumed late pre-B-cell developmental stage (i.e., with L chain DNA rearrangement but no L chain expression) has not been clearly identified in

normal cell populations and may represent a transient stage of normal B cell development.

As the pre-B cell develops to an immature B cell, L chains are expressed and IgM monomers (H_2L_2) are displayed on the cell surface as antigen receptors (177). The immature B cell matures to a B cell that co-expresses IgM and IgD on the B lymphocyte surface (57). Co-expressed μ and δ chains in IgM and IgD, respectively, have the same V region, and their expression exhibits allelic exclusion (36, 64, 133). Up to this stage, B-lymphocyte development is antigen independent, although immature B cells that express surface IgM are presumably capable of being affected by contact with antigen or anti-idiotypic antibody. Maturation beyond the B cell stage is thought to require activation by mitogens or an appropriate combination of antigen, T cells or T cell factors, and macrophages (100a, 109a). The activated B cell that secretes significant amounts of IgM matures into either a memory B cell that continues to express surface Ia antigens but expresses a new isotype of surface immunoglobulin (IgG, IgE, or IgA), or a terminally differentiated plasma cell that no longer expresses surface Ia antigens but expresses large quantities of secreted IgM, IgG, IgE, or IgA with little if any surface Ig (190, 122). Antigenic stimulation of memory B cells generates plasma cells secreting IgG, IgE, or IgA.

Either memory B cells or plasma cells can express immunoglobulin isotypes other than IgM or IgD. The expression of different H chain C regions (C_γ, C_ϵ, or C_α) occurs with the same V region associated with C_μ in IgM on the surface of immature B cells and is a result of a process called H chain "class switching." Heavy chain class switching is thought to be an antigen- and T cell-dependent event mediated by DNA rearrangements that delete the C_μ region and bring the H-chain V region next to a C_γ, C_ϵ, or C_α region gene segment (42, 71, 191, 192).

In addition to differential expression of H and L chains, there is differential expression of J chain during B-cell development (100). There is little or

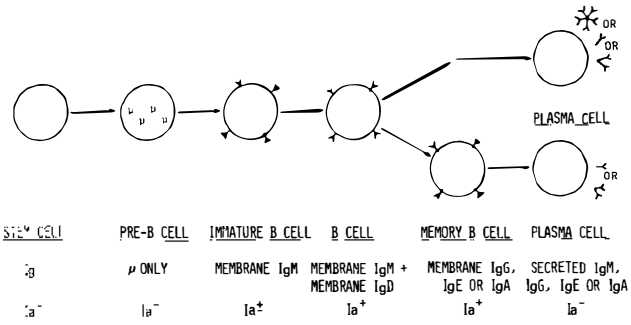

Figure 1 A simplified scheme of B-cell differentiation showing stages in immunoglobulin and Ia antigen gene expression (100a).

no J chain expression in the pre-B cell, immature B cell, or B cell stages of development, but J chain expression is prominent in all plasma cells and apparently occurs in all B cells that secrete immunoglobulins (100, 105a, 134). Similar to the situation for H chain and L chain expression, J chain expression is elevated substantially (ca 10- to 100-fold) if one compares cell lines that represent early and late stages of B cell development.

Tumors of the B cell lineage corresponding to various developmental stages have proven to be of great value in the analysis of immunoglobulin gene expression. The pre-B cell is represented by the carcinogen-induced 70Z cell line (122a), as well as by Abelson virus-transformed lymphoid cell lines (6, 142a). Immature B cells, B cells, and memory B cells are represented by B cell lymphomas (57, 92, 100a, 159, 180, 188). Plasma cells are represented by plasmacytomas (myeloma) tumors (132). Needless to say, it is critical to study normal cells from different B lymphocyte developmental stages to verify that cell lines and tumors represent valid models for B lymphocyte development. Although some studies of normal cells have been possible, isolation of pure populations of cells representing a particular B lymphocyte developmental stage previously has been difficult (155, 164). The recent development of continuous cloned lines of normal mouse lymphoid cells and their precursors is an extremely important breakthrough for future studies on B cell development (72, 183).

STRUCTURAL FEATURES IN IMMUNOGLOBULIN GENE CONTROL

Regulation of Immunoglobulin Gene Formation: Allelic and Isotypic Exclusion

Functional H chain and L chain expression is subject to allelic exclusion, in both normal B cells and B-cell tumors or tumor cell lines. Allelic exclusion is reflected in the random expression within a single cell of either the paternal or the maternal allele for each H or L chain. Although rare exceptions that may violate allelic exclusion have been reported for B-cell tumors, it is likely that allelic exclusion applies to greater than 99% of B cells. Allelic exclusion has not been described for other eukaryotic autosomal genes. In addition to allelic exclusion, functional L chain expression in normal B cells and B-cell tumors or cell lines is subject to isotypic exclusion, so that a single cell generally expresses either κ or λ L chains. Although some cloned murine B cell lines simultaneously express κ and λ L chains, it remains to be determined if both kinds of L chain are functional (3, 46, 92). As a result of allelic exclusion and L chain isotypic exclusion, a single B cell usually expresses functional immunoglobulin molecules (comprised of associated H chains and L chains) with only one combining site specificity.

As a consequence of the Dreyer & Bennett (45) hypothesis that immunoglobulin genes are created somatically by joining V and C gene segments, it was proposed that the creation of a joined V + C gene may generate a signal (perhaps the immunoglobulin chain itself) that inhibits subsequent joining of V and C genes in the same family (45, 141). The finding that cloned B cells can simultaneously express both normal and abnormal L chains, apparently encoded by two rearranged genes, required modification of this idea so that the signal that prevents further joining events is not activated by an abnormal joining event but only by a normal joining event. A large number of studies from many laboratories have resulted in substantial support for this modified hypothesis to explain allelic exclusion for both H and L chain as well as L-chain isotypic exclusion.

Analysis of L-chain genes in normal B cells, hybridomas, and B-cell tumors or cell lines representing different stages of B cell development can be briefly summarized: (*a*) In approximately two thirds of normal or transformed B cells producing κ L chains, one κ allele is functionally rearranged and one allele remains in a germline configuration; (*b*) in approximately one third of normal or transformed B cells, one κ allele is functionally rearranged and one allele is aberrantly (see below) rearranged or deleted; (*c*) with one exception, all κ alleles are deleted or aberrantly rearranged in normal or transformed B cells that express functional λ L chains but no functional κ L chain; (*d*) λ alleles remain in a germline configuration in B cells that express a functional κ L chain; and (*e*) λ alleles can be aberrantly rearranged in B cells that express a functional λ L chain (3, 37, 46, 84, 118). Aberrant rearrangements of κ (or λ) alleles can be of several types: (*a*) joining of a normal V gene segment to a non-J or pseudo-J segment: (*b*) joining of a pseudo-V or non-V segment to a normal J segment: and (*c*) joining of a normal V segment to a normal J segment so that codons are deleted from the VJ junction or an out-of-phase reading frame is established beyond the VJ junction (3, 7, 17, 22, 34, 46, 87, 90, 108, 129, 148–150). In addition, several possible examples (grouped with aberrant rearrangements above) of B cells express a normal κ (or λ) L chain and also express an abnormal second L chain despite an apparently correct VJ joining event (22, 148). Aberrantly rearranged L chain genes may be transcribed and translated, but they do not encode functional L chains (i.e. L chains that efficiently assemble to H chains for expression as membrane or secreted immunoglobulin).

These data on L-chain gene expression have led to two current hypotheses regarding L chain isotypic and allelic exclusion. First, it has been proposed that there is a hierarchy of L-chain gene formation in which κ alleles are rearranged before λ alleles. However, more complicated possibilities cannot be ruled out with certainty (e.g., κ genes are specifically deleted

or can undergo aberrant rearrangements in B cells that express a functional λ L chain). Second, Alt et al (3) have proposed that further L-chain gene rearrangements are actively prevented when a functional L chain (see above) is expressed. However, the possibility that a functional VJ junctional DNA or RNA sequence somehow provides the signal to prevent further L chain rearrangements cannot be rigorously disproved at present.

Although less is known about allelic exclusion of H-chain expression, some differences and similarities to L chain are apparent: (*a*) In 90% or more of normal or transformed B cells representing a variety of B cell developmental stages expressing H chains, both H-chain alleles are rearranged (presumably one functionally and the other aberrantly); (*b*) in 90% or more of Abelson virus-transformed null lymphoid cell lines, all detectable H chain alleles are rearranged (presumably aberrantly) or deleted, even though no functional H chain is expressed; (*c*) an Abelson virus-transformed lymphoid cell line that has undergone a potentially functional D-J_H joining event generates subclones with different function V_H-D-J_H combinations; (*d*) L-chain genes are never present in a rearranged form unless at least one H chain gene is rearranged; and (*e*) as noted for L-chain genes, transcription and translation of some aberrantly rearranged H-chain genes can occur (1, 5, 6, 29, 131, 140, 179, 193).

These results have led to the following proposals for regulation of H chain gene formation. First, H-chain genes are rearranged at an earlier stage of B-cell development than are L-chain genes. Second, formation of functional H-chain genes is much less efficient than is formation of functional L chain genes, possibly as a consequence of the more complex events involved (i.e. D → J_H plus V_H → D joining, with the possibility of D → D joining and somatic mutation as well). A significant population of null B lymphoid cells seems to result from the inefficient formation of functional H-chain genes (6). Third, functional H-chain gene formation seems to be necessary before further B-cell development and L-chain rearrangement can occur. Fourth, allelic exclusion of H chain may be an active process in which expression of a functional H chain prevents additional H-chain gene rearrangements. The possiblity that functional V_H-D-J_H junctional DNA or RNA sequences provide a signal for allelic exclusion seems unlikely on the basis of a recent report (5).

In summary, although the nature and hierarchy of H and L chain gene rearrangements are relatively well established, more direct proof of the hiearchy of κ and λ L-chain gene rearrangements and of allelic exclusion of immunoglobulin gene expression is needed. In addition, much remains to be learned about the molecular mechanisms and events that control these processes.

Transcriptional Control Signals in Immunoglobulin Genes

The initiation sites for both light and heavy chain transcription units exhibit "promoter" elements similar to these in other eukaryotic genes (reviewed in 25) located 5' to the leader coding sequence in V-region gene segments (Figure 2). These sequences function in phasing correct initiation of transcription (25). These sequences in immunoglobulin genes are not altered by somatic mutation, even though nearby V regions show up to 5% base changes (35). Thus, somatic mutations of V regions is apparently not a mechanism used in controlling levels of immunoglobulin gene transcription.

Enhancer sequences are a newly discovered class of control elements, which appear to affect the efficiency or level of transcription and which are located several hundred nucleotides upstream (i.e. to the 5' side) of the "TATA box" and cap site in the few viral and cellular genes where they have been identified (10, 11, 44, 61, 62, 94, 111, 165, 166). These so-called activator or enhancer sequences may be involved in RNA polymerase II recognition of eukaryotic transcription units in chromosomes, and appear to affect the efficiency of transcription (10, 11, 25, 44, 61, 62, 94, 111, 165, 166). Two features also suggest that these regions may be important for specific gene regulation. The first is the finding that these enhancer sequences exhibit no significant sequence homology, as would be expected for specific rather than universal gene control regions such as the TATA box. Second, in virus systems these enhancer sequences are functionally interchangeable (10, 44, 94) and their activity is correlated with host range restriction and the specificity of viral gene expression. Polyoma and SV40 mutants with altered host range from the wild-type virus have minor sequence alterations exclusively restricted to these specific regions (54, 63, 76, 152).

The enhancer sequences in viral genomes and in cellular histone genes are located some distance 5' of the TATA box and cap site (61, 62). Several lines of evidence raise the intriguing possibility that enhancer sequences in immunoglobulin genes might not be conventionally located in the flanking DNA 5' to the TATA box but instead are associated with the C region. First, immunoglobulin germline V regions contain 5' flanking sequences with all of the general signals (TATA box, cap site) needed for correct initiation of transcription (13, 18, 21, 75, 79, 108, 167), because specific initiation of transcription is observed in vitro and in *Xenopus* oocytes (13). Nonetheless, unrearranged V regions are not transcribed in vivo (106), whereas rearranged V + C genes are actively transcribed in myeloma cells (56, 104). Second, unrearranged C_κ regions are transcribed in myeloma cells at levels comparable to active rearranged light chain genes (173). Tran-

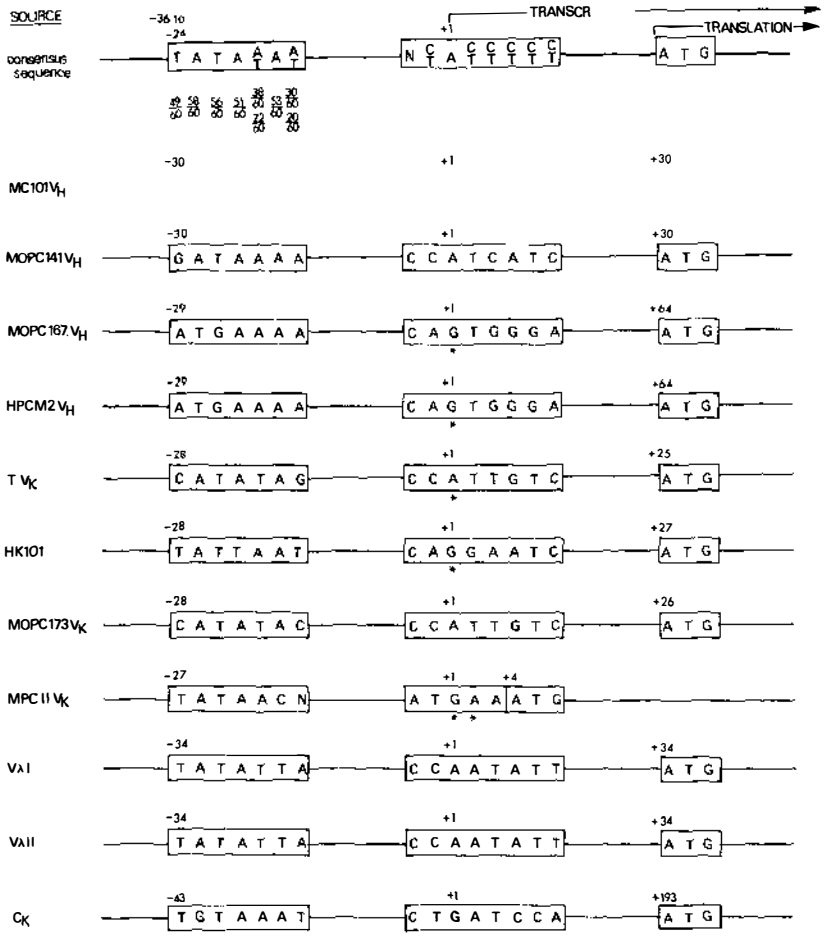

Figure 2 Comparison of the 5' flanking sequences of representative immunoglobulin V region gene segments. Selected immunoglobulin light and heavy chain sequences are aligned to indicate the conserved placement of presumed promoter sequences in relation to the consensus sequence of Breathnach & Chambon (25). Experimentally determined transcription initiation sites (i.e. cap sites) are denoted by asterisks. The other immunoglobulin sequences shown are aligned on the basis of homology with experimentally established transcription initiation sites. References for V region 5' flanking sequences are MC101V_H and MOPC141V_H (75), MOPC167V_H and HPCM2V_H (35), T V_κ (21), HK101 (13), MOPC173V_κ (108), MPC11V_κ (79), $V_{\lambda I}$ (18), $V_{\lambda II}$ (167), C_κ (173).

scripts of the unrearranged C_κ-gene segment begin near a sequence strongly resembling a TATA box. This strongly suggests that the C_κ (and possibly other immunoglobulin C regions) may contain an enhancer-like sequence. Finally, sequence comparisons of mouse and human J + C_κ germline gene

segments have revealed a region of strikingly conserved sequence homology approximately 0.7 kb 5' to the C_κ region (68). This is striking because the only other areas of sequence conserved between the mouse and human genes are the J and C_κ coding regions. Interestingly, we have noted that this highly conserved intron sequence 5' to the C_κ region contains short sequences related to the SV40 enhancer sequences and to the specific polyoma enhancer sequences from polyoma host range variants (R. Deans, R. Wall, personal communication). This conserved intron sequence is present in an aberrantly rearranged κ light chain gene (from MPC 11), which is the smallest active immunoglobulin transcription unit known. This truncated κ gene is as actively transcribed as the normally rearranged V + C_κ gene in MPC 11 cells (34, 150). Heavy chains also appear to contain control sequences in the $J_H \rightarrow C_H$ intron sequence (5, 35, 80, 81). Interestingly, large deletions in this intron region in 18–81 pre-B cell lines are correlated with the loss of μ expression, which is restored by LPS stimulation (4). If this novel prediction is confirmed, then the V region (with the general promoter elements essential for correct transcript initiation) and the C region (with enhancer function) both contribute important functions for activating immunoglobulin genes.

Methylation and Gene Expression

IMMUNOGLOBULIN GENES

The demethylation of methylated cytosines in chromosomal DNA is closely correlated with gene expression as in many eukaryotic gene systems (reviewed in 134a, 139, 185). Most of the methylation in mammalian DNA is 5-methylation of cytosines in the symmetrical dinucleotide C-G, and most of the C-G sequences contain 5-methylcytosine (C^m-G). The sequence C-C-G-G is cut by restriction enzymes Hpa II and Msp I, whereas C-C^m-G-G is cut only by Msp I. Therefore, parallel Southern blots (161) of chromosomal DNA cut with HpaII or Msp I can be used to show whether these restriction sites are methylated or not.

Methylation patterns have been analyzed in mouse heavy chain genes (C_μ, C_δ, and $C_{\gamma 1}$, $C_{\gamma 2b}$) that are activated by different mechanisms. The C_μ gene is expressed as a result of the DNA rearrangements that join the V_H, D_H, and J_H gene segments (V region formation) (reviewed in 46). The C_δ gene is expressed by transcription through from the rearranged C_μ gene, in a VDJ_H-C_μ-C_δ complex transcription unit (33, 102, 112). The $C_{\gamma 1}$ and $C_{\gamma 2b}$ genes are expressed by means of DNA rearrangements, which bring the VDJ_H close to either the $C_{\gamma 1}$ or $C_{\gamma 2b}$ gene segment (class switching) (reviewed in 46, 103).

The HpaII/MspI restriction mapping technique has been used to determine the methylation of immunoglobulin heavy chain genes. The C_μ, C_δ, $C_{\gamma 1}$, $C_{\gamma 2b}$, and C_α regions all are methylated in cells that do not express them but that are demethylated when they are expressed (39, 139, 190). In particular, the δ gene remains methylated, and thus presumably untranscribed, in a lymphoma cell line that probably represents an early stage of B cell differentiation and produces only μ heavy chains (139). Because μ and δ RNAs are co-transcribed from a single complex transcription unit at a later stage of B-cell differentiation, this finding suggests that the μ-plus-δ-complex transcription unit is of variable length regulated by C_δ methylation.

These results show complete correlation between demethylation and expression of immunoglobulin C_H genes, in agreement with results in numerous other eukaryotic gene systems (reviewed in 134a, 139, 185, 190).

Selective demethylation of one heavy chain C-region allele represents one possible molecular mechanism for allelic exclusion. However, in the B cell lymphoma W279, which makes only a single allotype of μ chain (153a, 180), both alleles of the C_μ gene are demethylated. Furthermore, both $C_{\gamma 1}$ regions are also fully demethylated in IgG-secreting P3K myeloma cells (139). If these findings in established tumor cell lines are relevant to regulation in normal B cells, it would appear that selective demethylation of one chromosome is not the mechanism of allelic exclusion.

J-CHANGING

Secreted pentameric IgM (and multimeric IgA) is assembled by covalent linkage to a small protein called the J chain. J-chain synthesis is induced in B cells after antigenic stimulation (85, 100). Its production is initiated by the onset of J-chain gene transcription (105a). J-chain genes are not rearranged when germline DNA is compared to DNA from lymphoid cells expressing J chain. The J-chain gene is methylated in tumor cell lines representative of pre-B cells; immature and mature B-lymphocytes do not express J chain. However, the J-chain gene is demethylated in immunoglobulin-secreting cell lines representative of plasma cells (190). J-chain expression is thus closely correlated with demethylation, just as are immunoglobulin genes.

It is not clear whether or not demethylation precedes immunoglobulin gene and J-chain gene transcription and is a mechanism that initiates expression. However, recent studies showing that estrogen induction causes demethylation of the 5'-flanking regions of hen vitellogenin (185) and that in vitro methylation of a single HpaII site in SV40 inhibits late but not early

SV40 expression (53) strongly support the likelihood that demethylation (at least in 5' control regions) precedes the onset of transcription.

Chromatin Structure in Immunoglobulin Gene Expression

As first demonstrated by Weintraub & Groudine (181), genes in chromosomes that are expressed (i.e. active chromatin) are preferentially digested by treatment of nuclei with the sequence nonspecific nuclease DNase I. Subsequent studies in a number of eukaryotic gene systems have confirmed that transcribed regions, as well as considerable flanking sequences of expressed genes, are more sensitive to nuclease digestion than are unexpressed genes or bulk chromatin (reviewed in 99, 107). Because immunoglobulin genes undergo both productive and aberrant DNA rearrangements, it is of interest to compare Ig gene expression and chromatin configuration in germline and various rearranged states in different lymphoid cells. Storb et al (163) have examined DNase I sensitivity of light-chain nuclear V_κ and C_κ gene segments in nuclei from myeloma cells (κ^+), B-lymphoma cells (κ^+), and liver cells (κ^-). They found that C_κ genes are sensitive to DNase I in both rearranged and germline configurations in myeloma tumors and a B lymphoma. Unrearranged V_κ regions are insensitive to DNase I, but become DNase I-sensitive when rearranged. No difference in DNase I sensitivity could be detected between the two rearranged C_κ regions of MOPC-21. In this cell line, one C_κ gene is productively rearranged and makes functional κ light chains; the other C_κ gene is aberrantly rearranged with a misalignment of V-J, which leads to a shift in translation reading frame (177a). This nonproductive C_κ gene is transcribed into a nonfunctional κ mRNA.

Restriction enzyme digestion of nuclei has shown that rearranged expressed C_κ regions are relatively more accessible to enzyme than are germline C_κ regions (130).

Many expressed eukaryotic genes in nuclei also contain sites (often in the flanking sequences 5' to the TATA box) that inhibit nuclease sensitivity 10-fold greater than transcribed gene sequences, which in turn are 10-fold more sensitive than is bulk chromatin (reviewed in 49, 99, 107). These so-called hypersensitive sites are apparently nucleosome-free in viral minichromosomes, and include the enhancer control regions important in regulating transcription (63, 73, 145, 174, 175). Furthermore, nuclease-hypersensitive sites in certain eukaryotic chromosomal genes contain DNA sites whose methylation pattern is correlated with tissue-specific gene expression (70, 99).

Chromatin from T lymphocytes from thymus exhibits a DNase I hypersensitivity in the sequences between C_μ and J_H (162). This may be related to the widespread occurrence of aberrant μ RNA species in T cells. These μ RNAs do not contain V_H regions and appear to initiate 5' to the J_H

region, where Clarke et al (35) have evidence for a transcription origin in unrearranged μ genes from certain myeloma cells.

Parslow & Granner (124) have recently determined the DNase I hypersensitivity patterns of the C_κ regions in the pre-B-cell line, 70Z (124). The 70Z cells only synthesize detectable κ mRNA with LPS (128), even though they contain a productively rearranged V + C_κ gene (in addition to an unrearranged C_κ region) (101a). LPS activation of κ mRNA transcription was associated with the appearance of a DNase I hypersensitive site approximately 0.7 kb 5' to the C_κ region exon (124). This sequence exhibits the hypersensitivity of known enhancer regions and coincides with the highly conserved sequence in the J → C_κ introns of mouse and human C_κ regions (68). This study found no evidence of hypersensitive sites 5' to the V region in the LPS-activated light chain gene. This interesting result further supports the proposal that enhancer sequences in immunoglobulin genes might be adjacent to the C regions. Weischet et al (182) have reported that DNase I hypersensitive sites are present in the 5' sequences upstream of both transcribed and untranscribed rearranged light chain genes in a myeloma cell.

CONTROL OF IMMUNOGLOBULIN GENE EXPRESSION BY RNA PROCESSING

Membrane and Secreted Heavy Chains

Membrane and secreted immunoglobulin heavy chains are coded by heavy chain mRNA species with different 3' ends generated by RNA processing mechanisms (reviewed in 140, 177). This was first shown for μ mRNA. Various B lymphoma cells and myeloma cells were found to contain two prominent μ mRNA's at 2.7 and 2.4 kb, which coded for membrane (μ_m) and secreted (μ_s) chains (2, 80, 128, 136, 158). Analysis of μ cDNA clones of both μ mRNA species revealed that the μ_s and μ_m mRNA's were identical through the end of the $C_\mu 4$ constant region coding sequence, but thereafter contained very different COOH-terminal coding segments and 3'-untranslated regions (3'-UT) (2, 136). The μ_s COOH-terminal sequence encoded a 20-residue hydrophilic segment identical to the μ_s COOH-terminal amino acid sequence determined by Kehry et al (1978). The μ_m COOH-terminal sequence encodes 41 residues (designated the M or membrane region) with the properties of a transmembrane protein (48, 136).

The locations of the COOH-terminal segments of μ_m or μ_s mRNA's were established by R-loop electron microscopy and nucleotide sequencing of the cloned μ gene (48, 136). The secreted COOH-terminus coding sequence and the 3' untranslated region of μ_s mRNA is encoded contiguous with 3' end of the $C_\mu 4$ domain (Figure 2). The μ_m COOH-terminus and 3'-UT region is encoded in two exons (the M exons) located 3' to the

$C_\mu 4$ domain. These two M exons are joined to the $C_\mu 4$ domain by two RNA splices that replace the μ_s COOH-terminus and 3'-UT region sequences to generate μ_m mRNA. The chromosomal gene codons at the 3' end of the $C_\mu 4$ domain where the M exons are spliced have the sequences G/GTAAA encoding Gly-Lys. This sequence in the μ chromosomal gene (30, 48) is identical to the consensus sequence for an "upstream" RNA splicing site, G/GTAAG (93, 138, 151). The underlined GT has been universally found at the exon/intron juncture of "upstream" splicing sites in eukaryotic genes (reviewed in 25).

Because all immunoglobulin heavy chains contain the sequence Gly-Lys at the end of the last domain encoded by a potential site for RNA splicing (G/GTAAA or G/GTAAG), Rogers et al (136) predicted that all other immunoglobulin heavy chain genes would also contain M gene segments and would generate membrane mRNA species by RNA splicing. It has now been confirmed that all B-cell lines that produced either γ_1, γ_{2a}, γ_{2b}, or γ_3 secreted heavy chains contained both γ_s mRNA and a minor γ mRNA species (γ_m) with a spliced structure analogous to that of the μ_m mRNA (135, 137, 140, 171). Mapping and sequencing of the γ_1, γ_{2a}, γ_{2b}, and γ_3 M gene segments (136, 171, 193) have located the M exons in these heavy chain genes. Membrane and secreted forms of α heavy chain mRNA have recently been reported (82, 189). The M exon of the α heavy chain gene segment has now been determined (189).

The C_δ gene is organized differently from other heavy chain genes in that it contains an extended hinge and lacks an internal C_H2 domain (98, 169). Unlike all other heavy chain genes where the secreted C-terminal sequences are continuous with the final C region domain, the δ gene has both its secreted and membrane exons in separate noncontiguous gene segments 3' to the $C_{\delta 3}$ domain (33, 98, 102, 112, 169, 170). The M exon sequences (33) of δ_m chains also exhibit a relatively high degree of homology to the M exon sequences of other heavy chains (140).

Mouse B lymphoma cells co-expressing membrane μ and δ chains contain both μ_m and μ_s mRNA, as well as two δ_m mRNA species (102, 112, 116). Both these δ_m mRNA species apparently contain identical coding sequences but terminate at different poly(A) sites and therefore have different 3'-UT sequences (33, 102). An analogous situation has been reported for two H-chain α_m mRNA species for α heavy chains (189) and dihydrofolate reductase (153). There is no evidence for functional differences between these two forms of δ and α mRNA's.

Heavy Chain Genes are Complex Transcription Units

Most eukaryotic genes now studied (including immunoglobulin light chain genes) (127, 178) are simple transcription units that code for a single mRNA (reviewed in 40, 41). Simple transcription units contain a single

poly(A) addition site, and the pattern of RNA splicing is invariant except in unusual cases where alterations in splicing sites generate aberrant splicing patterns. Complex transcription units contain multiple poly(A) addition sites and/or alternative RNA-splicing sites, which are used to generate multiple mRNA species from a single gene (40, 41, 197). Complex transcription units have been found in adenovirus, SV40, polyoma virus (40, 41, 197), and retroviruses (165, 166). The mapping of membrane and secreted mRNAs established immunoglobulin heavy chain genes as the first-known nonviral complex transcription units. The genes for yeast invertase (31) and rat calcitonin (8) have recently been shown to be complex transcription units. Immunoglobulin heavy chain complex transcription units contain at least two poly(A) addition sites. When nuclear RNA precursors have poly(A) added at the end of the secreted COOH-terminal sequence, RNA splicing then acts on all exons to yield a secreted heavy chain mRNA (Figure 3). Nuclear RNA precursors that have poly(A) added at the end of the M exons undergo two additional RNA splices that delete the secreted C-terminal sequence and connect the final C region domain to the M exons to generate membrane heavy chain mRNA's.

Even though membrane heavy chain mRNAs are produced by two RNA splicing events more than are required for secreted heavy chain mRNAs, it is believed that the processing pathways leading to secreted or membrane heavy chain mRNA's are determined by the choice of poly(A) addition sites (48, 136, 140, 178). This proposal is based on the general finding that polyadenylation precedes splicing under normal conditions (reviewed in 40, 41).

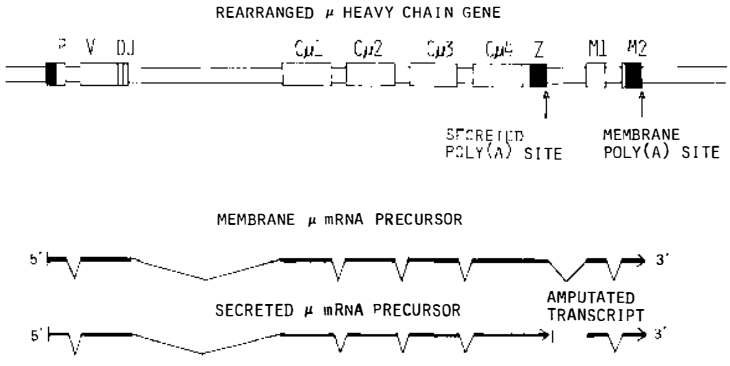

Figure 3 RNA species and splicing events in the generation of membrane and secreted heavy chain mRNA. The upper line depicts the structure established for the μ gene (30, 48). Boxes are exons and untranslated regions are shaded. The lower two lines depict the two polyadenylated alternative primary transcripts made from a single gene, with their splicing patterns. The amputated transcript is a novel polyadenylated RNA species generated in the addition of poly(A) to the secreted mRNA precursor. Although shown here for the μ gene, all heavy chain genes have a similar organization encoding membrane and secreted mRNA species.

Several studies have reported large nuclear RNA precursors of sizes expected for primary transcripts and nuclear RNA processing intermediates to secreted and membrane heavy chain mRNA's (35, 104, 128, 140, 147). However, because of the low levels of the presumptive primary transcripts and difficulties in reproducibly detecting splicing intermediates, the differential processing pathways leading to membrane and secreted heavy chain mRNA's have not been resolved. However, Rogers & Wall (137, 140) have obtained another line of evidence in support of the proposal that membrane and secreted heavy chain mRNA's are processed from the transcripts of a complex transcription unit. As a general rule, it appears that poly(A) addition occurs at points of cleavage in nuclear RNA molecules rather than through termination of transcription (40, 69, 117). Indeed, transcription apparently continues some distance past poly(A) addition sites (several kilobases) before cleavage of the nascent transcript occurs. Assuming that poly(A) addition occurs at points of cleavage in immunoglobulin heavy chain gene transcripts, it was reasoned that transcription past the poly(A) addition site for secreted mRNA precursors would produce a novel polyadenylated species called the "amputated transcript." This predicted polyadenylated RNA species should contain intron sequences between the secreted poly(A) site and the membrane exons (Figure 2). Since the M1 and M2 exons are separated by complete splicing signals in the amputated transcript, it is likely that this intron would be spliced out. Finally, the amputated transcript should have a 5' terminus beginning in the sequence following the secreted poly(A) addition site and should not contain any heavy chain V or C_H region exons. Discrete nuclear RNA species with precisely these predicted properties have now been detected and characterized in both μ- and γ_{2b}-producing cells (137, 140). The confirmation of the amputated transcript as predicted establishes that heavy chain genes are complex transcription units, and reaffirms that poly(A) is added by a mechanism involving RNA cleavage.

Co-Expression of Different Heavy Chain Classes

It was predicted that the co-expressed μ and δ heavy chains in surface IgM and IgD would be generated from a single large complex transcription unit by RNA processing mechanisms (55, 178). This prediction was based on the findings that co-expressed μ and δ chains appeared to have the same variable region (reviewed in 57), exhibited allelic exclusion, and were encoded on the same chromosome (23, 64). The C_μ and C_δ regions are closely linked. The C_δ gene is separated from the μ_m exons only by approximately 2 kb of DNA (33, 98, 102, 112, 169).

If μ and δ are co-expressed from a single gene, then the δ gene should not be rearranged in IgM + IgD-producing lymphocytes. This prediction

was confirmed by mapping studies that showed that the C_δ gene was in the germline configuration in $\mu + \delta$-producing cells (83, 102, 112). These data clearly indicated that IgD expression in $\mu + \delta$ B cells did not involve a V_H to C_δ DNA class-switch DNA rearrangement. Accordingly, the simultaneous expression of C_μ and C_δ with a single V_H region appears to be mediated by alternative routes of RNA processing of a very large primary nuclear transcript that contains the V_H, C_μ, and C_δ gene segments (reviewed in 102, 140).

The $\mu + \delta$ complex transcription unit is approximately 25 kb long, with five known alternative polyadenylation sites and more than a dozen potential exons for splicing. Large nuclear RNA species approaching the 25-kb size estimated for the $\mu + \delta$ primary transcript have eluded direct detection. These RNAs are likely to be present in <10 copies/cell, and this low abundance may preclude their direct detection. A complete primary transcript for the major late adenovirus transcription unit (32 kb long) was never detected directly (40, 41, 197). Instead, confirmation of this major transcription unit was obtained by indirect means, including pulse-labeling studies and UV transcript mapping (40, 41). Such indirect means appear likely to be required for confirming the large $\mu + \delta$ complex transcription unit. As in heavy chain transcription units for membrane and secreted mRNA's, the choice of poly(A) sites in the $\mu + \delta$ transcription unit is presumed to determine the pattern of RNA splicing. The RNA splicing events in this complex system are more complicated than in membrane and secreted heavy chain mRNA processing where all complete RNA splicing sites are used. When the V_H region is spliced to the C_δ region to make δ mRNA, RNA splice sites in the $C\mu$ exons are apparently ignored.

How C_μ or C_δ exons are chosen for splicing remains a mystery. A model for RNA splicing has been proposed in which U-1, a ubiquitous small nuclear RNA in eukaryotic cells, base-pairs with both ends of an intron and aligns them precisely in register for cutting and splicing (93, 138). Yang et al (195) have obtained preliminary evidence in support of this model. However, this model does not explain how μ exon splice sites that function in making μ mRNA's are ignored in splicing δ mRNA's. It seems plausible that the secondary structure of the nuclear RNA molecule might direct the course of RNA splicing, but this hypothesis is not yet amenable to experimental testing.

IgD-secreting myeloma cells contain a single prominent δ_s mRNA species (52, 102, 112, 116). Rare instances of IgD secretion by myeloma cells in mice apparently do not occur through RNA splicing, but rather appear to result from a DNA rearrangement that brings the rearranged VDJ_H region into proximity to the C_δ gene with the deletion of the C_μ gene segments (102, 112). Furthermore, the δ_s mRNA is not detectable in B

lymphomas, making μ_s, μ_m, and the two δ_m mRNA species. These findings indicate that two different mechanisms are employed in δ gene expression and the production of IgD. RNA processing alterations in B cells produce membrane δ mRNA's along with μ mRNA's from a single large complex transcription unit, whereas a rare DNA rearrangement in mouse plasma cells produces secreted δ mRNA.

Rare lymphoid cells have been reported that apparently contain two classes of surface immunoglobulins other than IgM and IgD. These cells are of considerable interest because they may represent memory B cell transitional stages in the process of class switching, leading to antibody secretion (reviewed in 103). Such cells have recently been isolated and their immunoglobulin gene organization has been analyzed. Perlmutter & Gilbert (125) have shown that splenic B lymphocytes exhibiting surface IgM and IgG1 contain two C_μ genes and $C_{\gamma 1}$ genes that show no evidence of rearrangement. Similarly, $\mu^+\epsilon^+$ splenic B lymphocytes isolated by fluorescence-activated cell sorting show no evidence of DNA rearrangements between J_H and the C_ϵ gene segment (194). These two reports conclude that the co-expression of different H chain mRNA's involves extremely large nuclear RNA precursors. Both these reports are based on the presumption that the isolated B lymphocytes exhibiting two different heavy chains by staining are simultaneously synthesizing both chains. This is partially satisfied for the $\mu^+\epsilon^+$ cells, but this point needs to be rigorously confirmed.

This reservation is not confronted in other studies on cloned B lymphocyte lines derived from an Abelson virus-transformed pre-B cell line called 18–81, which express two H chain classes. As characteristic for a pre-B cell, primary 18–81 subclones only express low levels of μ mRNA but no light chain mRNA. Long-term tissue-culture-adapted subclones of 18–81 (18–81 A-2) apparently undergo a switch from C_μ to $C_{\gamma 2b}$ expression (4, 28). The level of γ_{2b} mRNA appears to be inducible with a B cell mitogen, lipopolysaccharide. Furthermore, μ chain expression is undetectable without LPS but is restored by LPS treatment of these cells. These cells contain C_μ regions. Deletion events occurring within the J_H-C_μ intron are correlated with the loss of constitutive μ gene expression and the $\mu \rightarrow \gamma_{2b}$ switch (4). The $J_H \rightarrow C_\mu$ intron has been shown to contain repetitive DNA sequences that readily undergo deletion events (46, 103). These experiments have not shown that the δ, γ_3, and γ_1 gene segments are present in their germline context. If this is the case, the $\mu \rightarrow \gamma_{2b}$ switch in 18–81 A-2 cells may result from the differential RNA splicing of a large (>100 kb) multi-C_H-gene transcript (4). Given the large sizes of the predicted $\mu + \gamma_1$, $\mu + \epsilon$, or $\mu + \gamma_{2b}$ nuclear RNA precursors (100–200 kb), and the experimental inability to detect even the 25-kb $\mu + \delta$ co-transcript, experimental confirmation of these large precursors will require ingenious approaches. It also remains to be established that such cells represent

transitional stages in heavy chain class switching. Immunoglobulin gene introduction into lymphoid cells together with defined conditions that induce class switching (mitogens, T cell factors, etc) should provide further insights into the molecular mechanisms and dynamics of class switching.

QUANTITATIVE CHANGES IN LEVEL OF IMMUNOGLOBULIN EXPRESSION DURING DEVELOPMENT

As noted above, there is differential expression of H, L, and J chains during development. In addition, the expression of all three products is amplified substantially during B cell maturation. In general, when both H and L chains are expressed (i.e. in immature B cells and later stages of development), they are synthesized at roughly equimolar levels even as the levels of expression are increased several orders of magnitude (86, 101, 105a, 113, 128, 134, 153b, 154). In contrast, the level of J chain expression is not tightly coordinated with the level of H and L chain expression, despite the fact that in plasma cells, J chain is expressed at levels roughly comparable to the levels of H and L chain expression (85, 100, 134). Cloned murine pre-B-cell lines and some immature B-cell lines may synthesize as little as 0.01–0.1% of their protein as μ only or as μ plus L chains (101, 153b, 154), whereas murine myeloma cells may synthesize as much as 20–30% of their protein as H and L chains (86, 113). Similar amplification of H and L chain expression in late stages of B lymphocyte development is also seen in studies of normal cells (109, 155,). The levels of H and L chain expression in most immature B-cell, B-cell, and memory B-cell lines appear to be roughly intermediate between the extremes cited above, although there are few critical measurements for the intermediate stages of B-cell development (100, 134, 188).

Various B-cell lines and normal B cells have been shown to amplify both H and L chain impression approximately two to ten times when stimulated in vitro with mitogens (e.g. lipopolysaccharide), anti-immunoglobulins, or appropriate combinations of antigen and lymphocyte supernatants (24, 60, 101, 109, 122b, 154). A number of pre-B cell lines stimulated with LPS begin to express L chain at levels comparable to H chain, with little stimulation of the level of H chain synthesis (101, 122b, 142a). In contrast, after LPS stimulation, several Abelson virus-transformed pre-B-cell lines are stimulated to synthesize H chains at an approximately 10-fold increased level but still do not express L chains (4, 5). It has been noted for these Abelson virus-transformed pre-B-cell lines that LPS stimulation of H-chain synthesis occurs only in sublines or clones that spontaneously have decreased the level of H-chain synthesis substantially (about 10-fold). The

decrease in the unstimulated level of H-chain synthesis is correlated with a substantial deletion in the J_H-C_μ intron 5' of the μ switch region. Based on these results, it has been suggested that LPS stimulation somehow compensates for the loss of a DNA sequence that has a positive effect on H-chain production. Myeloma cells with smaller deletions in the J_H-C_μ intron seem to be unaffected in their level of H-chain expression.

Somatic cell hybrids between mouse myeloma cells and mouse or human pre-B cells, immature B cells, B cells, or memory B cells co-dominantly express H and L chains at approximately the same high level of expression observed in the myeloma fusion partner. In all reported studies of this type, the overall phenotype of immunoglobulin expression (i.e. quantity of H and L chains and secreted versus membrane form of H chain) is determined by the more differentiated myeloma cell fusion partner (50, 91, 96, 134, 134b, 188).

A number of studies with murine cell lines have demonstrated that the qualitative and quantitative levels of H, L, and J chain expression at different stages of B cell development are determined largely, if not entirely, by the amount of cytoplasmic H, L, and J chain mRNA's, respectively (38, 56, 104, 128, 134, 147). The few studies on normal B lymphocytes are consistent with this conclusion (168).

The rates of in vivo H and L chain RNA synthesis and processing has been studied extensively in MOPC 21 and MPC 11 murine myeloma cell lines by the laboratories of Wall and Perry, respectively (56, 104, 147). These studies, together with other studies on the turnover of cytoplasmic mRNA in MOPC 21 myeloma cells (38), provide insight into how a single functional H- or L-chain gene can account for as much as 10% of the protein synthesized by a rapidly growing myeloma cell. The following conclusions apply to both myeloma cell lines: (*a*) The rate of transcription is greater than 20–30 transcripts per minute (which is comparable to the apparent maximal rates of transcription observed for ribosomal RNA); (*b*) processing of the putative primary transcripts into mRNA and transport of mRNA from the nucleus to cytoplasm is rapid and essentially quantitative; (*c*) cytoplasmic mRNA is very stable, so that it is minimally metabolized during the 20-hr cell generation time; and (*d*) the fraction of cellular mRNA encoding H and L chains is comparable to the fraction of cellular protein synthesis comprised by H and L chains. Thus, it appears that the level of H- and L-chain gene expression in myeloma cells may well approach the maximum level possible for a higher eukaryotic cell, with an approximate 20-hr generation time.

We know much less about regulatory steps that result in a 10- to 1000-fold lower expression of H and L chain mRNA (and protein) in B cells representing earlier stages of B lymphocyte development. However, some preliminary information is available from a study comparing steady-state

nuclear and cytoplasmic H chain mRNA contents of MPC 11 murine myeloma cells and the 70Z murine pre-B cell line (128). MPC 11 cells contain about 30,000 copies of H chain mRNA or about 300 times as much cytoplasmic H-chain mRNA as 70Z cells, consistent with the fact that H chain comprises about 10–12% and 0.02–0.10% of protein synthesis in MPC 11 and 70Z cells, respectively (86, 101). In contrast to the large difference in cytoplasmic H-chain mRNA content, only six times as much nuclear H-chain mRNA occurs in MPC 11 myeloma cells as in 70Z cells. The 50-fold higher ratio of nuclear to cytoplasmic H-chain mRNA in 70Z cells compared to myeloma cells is probably a consequence of differences in one or more of the following post-transcriptional events: (*a*) rate of H-chain nuclear RNA precursor processing; (*b*) extent of intranuclear H-chain mRNA degradation; (*c*) rate of transport of mature H-chain mRNA from the nucleus to the cytoplasm; and (*d*) rate of cytoplasmic H chain mRNA turnover. It is of interest that the ratio of nuclear to cytoplasmic nonimmunoglobulin poly(A)-containing RNA in either 70Z or MPC 11 myeloma cells is essentially the same as the ratio of nuclear to cytoplasmic H-chain mRNA in the 70Z cells. In view of the apparent differential post-transcriptional events specifically affecting H-chain mRNA metabolism, it seems unlikely that differences in transcription rates are fully responsible for the vastly different levels of cytoplasmic H-chain mRNA in these two cell lines. Obviously more studies of the transcription rates and metabolism of H- and L-chain mRNA's are required to understand how the levels of H- and L-chain mRNA (and thus H and L chains) are regulated during B lymphocyte development.

TRANSLATIONAL AND POST-TRANSLATIONAL REGULATION OF IMMUNOGLOBULIN EXPRESSION

Initiation of translation of H- and L-chain mRNA's appears to be much more efficient than the average cell mRNA in that H and L chains represent a larger fraction of the newly synthesized protein when there is a nonspecific decrease in initiation of translation (e.g. mitosis, starvation, viral infection) (119, 120, 160). However, there is no convincing evidence for regulation of the efficiency of mRNA translation in B cells of different developmental stages. Microsomal localization of H, L, and J chain mRNA's to the rough endoplasmic reticulum and translocation of H, L, and J chains into the cisterna of the rough endoplasmic reticulum seems to be determined by the amino terminal signal sequence, which is proteolytically removed from the primary translation product prior to chain termination and release from the ribosome (reviewed in 86). The signal sequence functions normally even in

an unusual situation where the signal sequence is contiguous with the C_κ sequence rather than a V_κ sequence, as found for normal κ L-chain mRNA's (51, 141). There are no known examples of either cell variants or normal regulatory events that alter binding of mRNA to the rough endoplasmic reticulum or the vectorial transport of immunoglobulin chains into the cisterna.

Glycosylation of all normal H chains as well as L chains that contain the Asn-X-Thr (or Ser) tripeptide N-glycosylation acceptor site in the amino terminal V region domain usually occurs by transfer of the core oligosaccharide (glucose$_3$-N acetyl-glucosamine$_2$-mannose$_{11}$) from a lipid carrier to a nascent polypeptide (15, 16). The transfer of the core oligosaccharide to a growing nascent H chain apparently slows polypeptide elongation sufficiently so that the net rate of H chain synthesis is decreased 10–25%, a phenomenon that may contribute to the slight imbalance of H and L chain synthesis observed in both normal and malignant B lymphocytes (9, 16, 86). Parameters that affect the efficiency of N-glycosylation or potential tripeptide acceptor sites of immunoglobulin chains are reviewed elsewhere (16). There is no evidence indicating that core glycosylation is regulated differentially in B cells of different developmental stages or in different physiological states. Most processing of the core oligosaccharide, as well as addition of terminal sugars, is thought to occur during the brief period the glycosylated immunoglobulin chain traverses the Golgi apparatus (16, 164). The decision process for generating simple versus complex asparagine-linked oligosaccharides is poorly understood. The best example of possible regulation of glycosylation involves a block in converting a core oligosaccharide to a complex oligosaccharide on the secreted H chains in less differentiated B cells (see below for a more complete discussion of this interesting regulatory event).

Intramolecular folding, including covalent disulfide bond formation, occurs to a significant extent on nascent L chains, and presumably on nascent H chains as well (14). Intermolecular assembly of immunoglobulin chains also begins on nascent H chains, with some classes of immunoglobulin forming H-H disulfide bonds and other classes of immunoglobulin forming H-L disulfide bonds on nascent H chains (15, 146). However, most assembly occurs between completed chains, with the pathway of assembly dependent on the class of Ig synthesized by the cell (19, 86, 146). Assembly of H chains is restricted to the chains of the same class, so that cells expressing μ and γ H chains, for example, do not form covalent μ-γ heterodimers (105).

The co-expression of secretory and membrane forms of H chain (e.g. μ_m and μ_s) in various ratios in the same cell at different B lymphocyte developmental stages raises the question as to whether or not the secretory

and membrane forms of H chains form heterodimers. The best studies to address this issue involve B lymphomas and myeloma cells, which simultaneously express γ_m and γ_s. In two different murine B lymphomas that express γ_{2am} and γ_{2as} in roughly equivalent amounts, no γ_{2am}-γ_{2as} heterodimers were seen (121, 188). Analysis of labeled cell surface Ig of MPC 11 myeloma cells revealed no detectable γ_{2bs} chains expressed on the cell surface (88). These results then suggest that membrane and secreted H-chain heterodimer covalent assembly is rare or non-existent in B cells where the membrane and secreted chains are co-expressed. Lack of significant heterodimer assembly of these chains could be due to one or more of the following: (*a*) preferential assembly of H chains synthesized by the ribosomes on the same mRNA molecule; (*b*) preferential assembly of H chains selectively partitioned into the membrane (H_m) instead of into the cisterna (H_s) of the rough endoplasmic reticulum; and (*c*) preferential assembly of homodimers due to intrinsic properties of the H chains. In contrast to the results cited above, recent work suggests that covalent heterodimer formation and membrane insertion of γ_{1s} and γ_{1m} H chains occurs in MOPC 21 myeloma cells, a cell line in which γ_{1s} is synthesized in a vast molar excess compared to γ_{1m} (58). This result must be questioned in view of the results in B lymphoma and other myeloma cells cited above. The putative γ_{1s} chains in labeled surface Ig of MOPC 21 cells could, for example, represent partially degraded γ_{1m} chains.

Differential intracellular degradation of mutant and normal H and L chains represents a well-documented example of post-translational regulation. For example, MOPC 173 and MOPC 21 myeloma lines synthesize a molar excess of L chains over H chains, but degrade the unassembled L chains (9). A MOPC 21 variant that synthesizes L chains but not H chains does not secrete the L chains, but quantitatively degrades them within the cell (184). Fusion of this MOPC 21 L chain-producing variant to a different myeloma cell permits assembly of the MOPC 21 L chain to a heterologous H chain. This assembly prevents the MOPC 21 L chain from being degraded intracellularly (184). However, in most lymphoid cells, unassembled L chains are not degraded intracellularly (9). Variant or normal cells that express H chain in the absence of L chain expression may or may not rapidly degrade the H chains within the cell (113, 154). Variant myeloma cells that express L chains and J chains, but not H chains, degrade the J chains (113–115). Finally, there is very little information regarding regulation of H, L, or J chain degradation in cells representing different B-lymphocyte developmental stages.

The final step in expression of Ig by B lymphocytes is cell surface insertion of membrane Ig or secretion of secreted Ig. In general, free L chains are secreted, whereas normal H chains are not secreted unless linked to L

chains (86, 113). Thus, pre-B cells generally do not secrete their H chains (101, 122b, 142, 154). A single report of human pre-B cells actively secreting H chain in the absence of L chain (95) needs to be rigorously documented by further experiments. In contrast, there are a number of examples of lymphoid cells that secrete mutant H chains in the absence of L chain expression (113). As noted above, unassembled MOPC 21 L chains are not secreted, but are degraded intracellularly. However, translation of MOPC 21 L chain mRNA in *Xenopus* oocytes results in an L chain that is neither secreted nor degraded (172). However, the MOPC 21 L chain is secreted if MOPC 21 H chain mRNA is simultaneously translated in oocytes (172). If another L chain MRNA is co-injected into oocytes with MOPC 21 mRNA, only the non-MOPC 21 L chain is secreted (172). Thus, it appears that the lack of secretion of free MOPC 21 L chain from MOPC 21 myeloma cells may not result from the rapid intracellular degradation of this L chain. Several variant myeloma cell lines synthesize mutant L chains or H chains that are not secreted even when assembled normally (113, 115). The mutations are not localized to a unique region. This suggests that these mutations may cause conformational changes in immunoglobulin chains that are incompatible with secretion.

Expression of membrane Ig seems to require simultaneous expression and co-assembly of H and L chains. Pre-B cells generally express cytoplasmic μ but no surface or secreted μ chains (95, 101, 122b, 153c, 154). There are several recent reports of murine and human pre-B lymphomas that express μ_m on the external cell surface in the absence of L chain expression (58a, 122a). The murine cell line (70Z) usually expresses cytoplasmic μ, but can express cell surface μ spontaneously or after dextran sulfate stimulation. If this result can be validated for normal B cells, it has important implications for potential regulation of pre-B cells by anti-idiotypic antibody or antigen.

A plethora of studies has attempted to determine whether glycosylation of normal or variant H chains is necessary for cell surface expression and/or secretion of Ig. In general, glycosylation of H chains is not necessary for cell surface expression (67, 153b). The results are more complicated for secretion of nonglycosylated Ig: (*a*) Secretion is blocked or markedly inhibited for tunicamycin-treated myelomas that synthesize IgM, IgE, and IgA, but a tunicamycin-treated hybridoma secreted a different IgA molecule at apparently normal rates; (*b*) secretion from tunicamycin-treated myelomas is little affected for IgG or IgD; (*c*) a B lymphoma (WEHI 279) secretes low levels of IgM at only a marginally slower rate after tunicamycin treatment (20, 65, 66, 82, 113, 153b, 155, 156, 157, 187). Some of the results obtained above do not depend on the cells per se, since the same hybrid cell is shown to secrete nonglycosylated IgG but not to secrete nonglycosylated IgM. In sum, whether or not nonglycosylated Ig is secreted depends on the class of Ig, the amount of Ig synthesized, and possibly the V_H gene segment

in the H chain. As noted above, there is no evidence for regulation of core glycosylation in B lymphocytes.

The most intriguing example of differential post-translational regulation of Ig at different stages of B cell development involves expression of H chains in secreted and surface Ig. As noted previously, B lymphocytes that synthesize H chain mRNA contain both membrane and secreted H-chain mRNA's. The ratio of secreted/membrane mRNA increases during B-cell development (140). From limited data, it appears that the ratio of intracellular membrane and secreted polypeptide chains reflects the ratio of the two H-chain mRNA's. Approximately 20–50% of the H chain is μ_s in pre-B or immature B cell lines and small resting B lymphocytes from animals (2, 4, 5, 48, 101, 136, 122b, 153b). Yet these cells secrete little (or none) of the μ_s polypeptide translation product (101, 122b, 155). In contrast, the μ_s translation product is quantitatively secreted from myeloma cells, B-cell lines and normal resting B cells stimulated with mitogens or T cell factors (86, 113, 122b). The lack of secretion of μ_s at early B cell developmental stages is not due to a lack of core glycosylation, since the unsecreted μ_s chains contain core oligosaccharide (but lack terminal sugars). Neither is it due to a block in H and L chain assembly, since IgM with μ_s chains is formed in these cells. Similar results have been found for a B cell line that expresses both α_s and α_m (82, 159), although the lack of secretion here is less well documented than the examples cited above.

The mechanism of this specific post-translational regulation of secretion is unclear. It might be due to any or a combination of the following: (*a*) differential oligosaccharide processing and terminal glycosylation of the core oligosaccharide; (*b*) failure of transport into the Golgi apparatus, which would explain the glycosylation deficiencies; (*c*) lack of J chain (100). The apparent lack of quantitative secretion of α_s from a B lymphoma argues against a role for J chain since IgA can be secreted as a monomer (82, 159). Similarly, the normal secretion of nonglycosylated IgM from a B lymphoma cell line (153b) argues that glycosylation does not explain this situation.

Studies on certain lines of 70 Z and 18–81 cells suggest that the expression of surface IgM may require more than the expression of μ_m and L chains (26, 122a, 142). The molecular mechanisms that control IgM secretion in early B cells represent interesting questions yet to be resolved.

FUTURE DIRECTIONS

The studies reviewed here clearly provide considerable insights into the events in immunoglobulin gene expression and regulation. Nonetheless, many of the molecular mechanisms affecting the changing patterns of immunoglobulin gene expression in B-cell development remain to be resolved.

The powerful resolution of recombinant DNA cloning and sequencing will continue to provide insights into immunoglobulin gene structure. Several exciting recent developments provide molecular immunologists even more penetrating approaches for dissecting immunoglobulin gene control. These include (*a*) the establishment of cloned normal pre-B cell and B cell lines that undergo developmental changes in culture (183a), (*b*) the successful introduction and expression of cloned immunoglobulin genes transfected into lymphoid cells (42a), and (*c*) the increasing refinement of culture conditions and factors that stimulate B cell growth and affect changes in immunoglobulin gene expression (55a, 93a, 124a).

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